

SEQUENCE LISTING**SEQ ID NO: 1**

Amino acid sequence of the Signal Peptide ER:

5

MKTNLFLFLIFSLLLSLSSAEF

SEQ ID NO: 210 Amino acid sequence of the Vacuolar targeting signal from Tobacco
chitinase A:

DLLVDTM

15

SEQ ID NO: 3

Nucleic acid sequence of the Forward primer:

cagaattcgcccccccctgca

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SEQ ID NO: 4

Nucleic acid sequence of the Reverse primer:

ctcagatcttggcgatgccaca

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SEQ ID NO: 5

Nucleic acid sequence of the forward primer from the 35S promoter:

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ctcagaagaccagaggct

SEQ ID NO: 6

Nucleic acid sequence of the backward primer from the terminator:

5 caaagcggccatcgtgc

SEQ ID NO: 7

10 Nucleic acid sequence of the human GCD cDNA used for the
constructs of the invention

gccccccc ctgcatccct aaaagcttcg gctacagctc ggtggtgtgt
 gcttgcaatg ccacatactg tgactccttt gacccccga cctttcctgc ccttgggtacc ttcagccgct
 atgagagtac acgcagtggg cgacggatgg agctgagtat ggggcccac caggctaatac acacggggcac
 15 aggcctgcta ctgaccctgc agccagaaca gaagttccag aaagtgaagg gatttggagg ggccatgaca
 gatgctgctg ctctcaacat ccttgccctg tcaccccctg cccaaaattt gctacttaaa tcgtacttct
 ctgaagaagg aatcggatat aacatcatcc gggtagccat ggccagctgt gacttctcca tccgcacct
 cacctatgca gacaccctg atgattcca gttgcacaac ttcagcctcc cagaggaaga taccaagctc
 aagatacccc tgattcaccg agccctgcag ttggcccagc gtcccgtttc actccttgcc agcccctgga
 20 catcaccac ttggctcaag accaatggag cggatgaatgg gaaggggtca ctcaaggagac agcccggaga
 catctaccac cagacctggg ccagatactt tgtgaagttc ctggatgcct atgctgagca caagttacag
 ttctgggcag tgacagctga aatgagcct tctgctgggc tgttgagtgg atacccttc cagtgcctgg
 gcttaccacc tgaacatcag cgagacttca ttgccctga cctaggtcct accctcgcca acagtactca
 ccacaatgtc cgcctactca tgctggatga ccaacgcttg ctgctgcccc actgggcaaa ggtggtactg
 25 acagaccag aagcagctaa atatgttcat ggcattgctg tacattggtg cctggacttt ctggctccag
 ccaaagccac cctaggggag acacaccgcc tgttccccaa caccatgctc tttgcctcag aggcctgtgt
 gggctccaag ttctgggagc agagtgtgcg gctaggctcc tgggatcgag ggatgcagta cagccacagc
 atcatcacga acctcctgta ccatgtggtc ggctggaccg actggaacct tgccctgaac cccgaaggag
 gaccaattg ggtgcgtaac tttgtcgaca gtccatcat tgtagacatc accaaggaca cgttttaca
 30 acagcccatg ttctaccacc ttggccactt cagcaagttc attcctgagg gctcccagag agtggggctg
 gttgccagtc agaagaacga cctggacgca gtggcactga tgcattccga tggctctgct gttgtggtcg

tgctaaaccg ctctctaag gatgtgcctc ttaccatcaa ggatcctgct gtgggcttcc tggagacaat
ctcacctggc tactccattc acacctacct gtggcatcgc cag

5 **SEQ ID NO: 8**

Glucocerebrosidase amino acid sequence

A R P C I P K S F G Y S S V V
C V C N A T Y C D S F D P P T F P A L G T F S
10 R Y E S T R S G R R M E L S M G P I Q A N H T
G T G L L L T L Q P E Q K F Q K V K G F G G A
M T D A A A L N I L A L S P P A Q N L L L K S
Y F S E E G V R L L M L N D Q R L L L P H W A K V
V L T D P E A A K Y V H G I A V H W Y L D F L A P A K A
15 T L G E T H R L F P N T M L F A S E A C V G S K F W E
Q S V R L G S W D R G M Q Y S H S I I T N L L Y H V V
G W T D W N L A L N P E G G P N W V R N F V D S P I I
V D I T K D T F Y K Q P M F Y H L G H F S K F I P E G S
Q R V G L V A S Q K N D L D A V A L M H P D G S A V V
20 V V L N R S S K D V P L T I K D P A V G F L E T I S P G
Y S I H T Y L W H R Q

SEQ ID NO: 9

25 ³⁵S Promoter nucleic acid sequence

Ttttcacaaagggtaatatcgggaaacctcctcggattccattgcccagctatctgtcattcatcg
aaaggacagtagaaaaggaaggtggctcctacaaatgccatcattgcgataaaggaaaggctatcgttca
agatgcctctaccgacagtgggtcccaaagatggacccccaccacgaggaacatcgtggaaaaagaaga
30 cgttccaaccacgtcttcaaagcaagtggattgatgtgatatctccactgacgtaagggatgacgcacaat
cccactatccttcgcaagaccttctctatataaggaagttcatttcatttgagaggac

SEQ ID NO: 10

Nucleic acid sequence encoding the ER signal peptide

5 atgaagactaatcttttctctttctcatcttttcacttctc ctatcattatcctcggccgaattc

SEQ ID NO: 11

Nucleic acid sequence encoding the vacuolar targeting sequence

10 gatcttttagtcgatactatg

SEQ ID NO: 12

15 Nucleic acid sequence of the terminator

taatttcatgatctgttttgtgtattcccttgcaatgcagggcctagggctatgaAtaaagttaatgt
gtgaatgtgtgaatgtgtgattgtgacctgaagggatcacgactataatcgtttataataaacaagactttg
tcccaaaaacccccccccngcaga

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SEQ ID NO: 13

Nucleic acid sequence of the expression cassette of the invention

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ttttcacaagggtaatatcgggaaacctcctcggattccattgcccagctatctgtcacttcatcg
aaaggacagtagaaaaggaaggtggctcctacaaatgccatcattgcgataaaggaaaggctatcgttca
agatgcctctaccgacagtggtcccaaatggacccccaccacgaggaacatcgtggaaaaagaaga
cgttccaaccacgtcttcaaagcaagtggattgatgtgatatctccactgacgtaagggatgacgcacaat
30 cccactatccttcgcaagacccttctctatataaggaagttcatttcatttggagaggacaggcttcttgag
atccttcaacaattaccaacaacaacaacaacaacattacaattactatttacaattacagtcga
gggatccaaggagatataacaatgaagactaatcttttctctttctcatcttttcacttctcctatcattatcc

tcggccgaattcggcccccctgcatccctaaaagcttcggctacagctcgggtggtgtgtgtctgcaatgcc
 acatactgtgactcctttgacccccgacctttcctgccccttggtaccttcagccgctatgagagtacacgca
 gtggcgacggatggagctgagatggggcccatccaggctaatacacacgggcacaggcctgctactgac
 cctgcagccagaacagaagtccagaaagtgaagggtttggaggggcatgacagatgctgctgctctc
 5 aacatccttgccctgtcacccccctgccccaaaatttgctacttaaactcgtacttctctgaagaaggaatcgga
 tataacatcatccgggtacccatggccagctgtgacttctccatccgcacctacacctatgcagacaccct
 gatgattccagttgcacaacttcagcctcccagaggaagataccaagctcaagatacccctgattcaccg
 agccctgcagttggcccagcgtcccgtttcactccttgccagcccctggacatcaccacttggtcaagac
 caatggagcggatgaatgggaaggggtcactcaaggacagcccggagacatctaccaccagacctgggc
 10 cagatactttgtgaagttcctggatgcctatgctgagcacaagttacagttctgggcagtgacagctgaaaa
 tgagcctctgctgggctgttgagtgatacccctccagtgctgggcttcaccctgaacatcagcgagac
 ttattgcccgtgacctaggtcctaccctcgccaacagtactcaccacaatgtccgcctactcatgctggatg
 accaacgcttgctgctgccccactgggcaaagggtggtactgacagaccagaagcagctaaatatgttcat
 ggcatgctgtacattggtacctggactttctggctccagccaaagccaccctaggggagacacaccgct
 15 gttcccaacaccatgctctttgcctcagaggcctgtgtgggctccaagttctgggagcagagtgtgaggta
 ggctcctgggatcgaggatgcagtacagccacagcatcatcacgaacctcctgtaccatgtggtcggctg
 gaccgactggaacctgcccgaacccgaaggaggaccaattgggtgcgtaactttgtcgacagtcca
 tcattgtagacatcaccaaggacacgtttacaacagcccatttctaccacctggccacttcagcaagt
 tcattctgagggtcccagagagtggggctggttgccagtcagaagaacgacctggacgcagtgccactg
 20 atgcatcccgatggctctgctgttggtcgtgctaaaccgctcctctaaggatgtgcctcttaccatcaagg
 atcctgctgtgggcttctggagacaatctcacctggctactccattcacacctacctgtggcatcgccaag
 atcttttagtcgatactatgtaatttcagatctgtttgttattcccttgcaatgcagggcctagggctatga
 Ataaagttaatgtgtgaatgtgtgaatgtgtgattgtgacctgaagggatcacgactataatcgtttataata
 aacaaagactttgtcccaaaaacccccccccngcaga
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SEQ ID NO: 14

Amino acid sequence of the recombinant protein of the invention

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M K T N L F L F L I F S L L L S L S S A E F A R P C
 I P K S F G Y S S V V C V C N A T Y C D S F D P P T F P

A L G T F S R Y E S T R S G R R M E L S M G P I Q A N
 H T G T G L L L T L Q P E Q K F Q K V K G F G G A M T
 D A A A L N I L A L S P P A Q N L L L K S Y F S E E G I G
 Y N I I R V P M A S C D F S I R T Y T Y A D T P D D F Q
 5 L H N F S L P E E D T K L K I P L I H R A L Q L A Q R P
 V S L L A S P W T S P T W L K T N G A V N G K G S L K G
 Q P G D I Y H Q T W A R Y F V K F L D A Y A E H K L Q
 F W A V T A E N E P S A G L L S G Y P F Q C L G F T P E
 H Q R D F I A R D L G P T L A N S T H H N V R L L M L
 10 D D Q R L L L P H W A K V V L T D P E A A K Y V H G I
 A V H W Y L D F L A P A K A T L G E T H R L F P N T M
 L F A S E A C V G S K F W E Q S V R L G S W D R G M Q
 Y S H S I I T N L L Y H V V G W T D W N L A L N P E G G
 P N W V R N F V D S P I I V D I T K D T F Y K Q P M F Y
 15 H L G H F S K F I P E G S Q R V G L V A S Q K N D L D
 A V A L M H P D G S A V V V V L N R S S K D V P L T I K
 D P A V G F L E T I S P G Y S I H T Y L W H R Q D L L V
 D T M

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Other Embodiments

It is to be understood that while the invention has been described in conjunction
 with the detailed description thereof, the foregoing description is intended to illustrate and
 not limit the scope of the invention, which is defined by the scope of the appended claims.
 Other aspects, advantages, and modifications are within the scope of the following claims.

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REFERENCES

1. Ma, J. K. C., Drake, P.M.W., and Christou, P. (2003) *Nature reviews* 4, 794-805
2. Lerouge, P., Cabanes-Macheteau, M, Rayon, C, Fischette-Laine, A.C., Gomord, V,
5 Faye, L. (1998) *Plant Mol Biol* 38, 31-48
3. Lee, R. E. (1982) *Prog Clin Biol Res* 95, 177-217
4. Grabowski, G. (1993) *Adv Hum Genet.* 21, 377-441
5. Grabowski, G. A., and Hopkin, R. J. (2003) *Annual Review of Genomics and
Human Genetics* 4, 403-436
- 10 6. Sorge, J. W., C., Westwood, B., Beutler, E. (1985) *Proc Natl Acad Sci USA.* 82,
7289-7293
7. Berg-Fussman, A., Grace, M., Ioannou, Y., and Grabowski, G. (1993) *J. Biol.
Chem.* 268, 14861-14866
8. Grace, M., Grabowski, GA. (1990) *Biochem Biophys Res Commun* 168, 771-777
- 15 9. Grace, M., Newman, K., Scheinker, V., Berg-Fussman, A., and Grabowski, G.
(1994) *J. Biol. Chem.* 269, 2283-2291
10. Barton, N. W., Brady, R.O., Dambrosia, J.M., Di Bisceglie, A.M., Doppelt, S.H.,
Hill, S.C., Mankin, H.J., Murray, G.J., Parker, R.I., Argoff, C.E., et al. (1991) *N
Engl J Med.* 324, 1464-1470
- 20 11. Grabowski, G. A., Barton, N. W., Pastores, G., Dambrosia, J. M., Banerjee, T. K.,
McKee, M. A., Parker, C., Schiffmann, R., Hill, S. C., and Brady, R. O. (1995) *Ann
Intern Med* 122, 33-39
12. Pastores, G. M., Sibille, A.R., Grabowski, G.A. (1993) *Blood* 82, 408-416.
13. Weinreb, N. J., Charrow, J, Andersson, H.C., Kaplan, P, Kolodny, E.H., Mistry, P,
25 Pastores, G, Rosenbloom, B.E., Scott, C.R., Wappner, R.S., Zimran, A. (2002) *Am
J Med* 113, 112-119
14. Bijsterbosch, M. K., Donker, W, van de Bilt, H, van Weely, S, van Berkel, T.J.,
Aerts, JM. (1996) *Eur J Biochem* 237, 344-349
15. Friedman, B., Vaddi, K., Preston, C., Mahon, E., Cataldo, J. R., and McPherson, J.
30 M. (1999) *Blood* 93, 2807-2816
16. Furbish, F. S., Steer, C.J., Krett, N.L., Barranger, J.A. (1981) *Biochim Biophys Acta*
673, 425-434

17. Doebber, T., Wu, M., Bugianesi, R., Ponpipom, M., Furbish, F., Barranger, J., Brady, R., and Shen, T. (1982) *J. Biol. Chem.* **257**, 2193-2199
18. Dwek, R. A., Butters, T.D., Platt, F.M., Zitzmann, N. (2002) *Nature reviews* **1**, 65-75
- 5 19. Neuhaus, J. M., Rogers, J.C. (1998) *Plant Mol Biol* **38**, 127-144
20. Vitale, A., and Galili, G. (2001) *Plant Physiol.* **125**, 115-118
21. Hellens, R., Edwards, EA., Leyland, N.R., Bean, S., Mullineaux, P.M. (2000) *Plant Mol Biol* **42**, 819-832
22. Wurtele, E. S., Bulka, K. (1989) *Plant Sci* **61**, 253-262
- 10 23. den Dulk-Ras, A., Hooykaas, P.J. (1995) *Methods Mol Biol.* **55**, 63-72
24. Laemmli, U. K. (1970) *Nature reviews* **227**, 680-685
25. Bradford, M. M. (1976) *Anal Biochem* **72**, 248-254
26. Stahl, P. G. S. (1982) *J Cell Biol* **93**, 49-56
27. Takasaki, S., Murray, G., Furbish, F., Brady, R., Barranger, J., and Kobata, A.
15 (1984) *J. Biol. Chem.* **259**, 10112-10117
28. Lerouge, P., Cabanes-Macheteau, M., Rayon, C., Fitchette-Laine, A. C., Gomord, V., and Faye, L. (1998) *Plant Mol. Biol.* **38**, 31-48
29. Frigerio, L., Pastres, A., Prada, A., and Vitale, A. (2001) *Plant Cell* **13**, 1109-1126
30. Frigerio, L., de Virgilio, M., Prada, A., Faoro, F., and Vitale, A. (1998) *Plant Cell*
20 **10**, 1031-1042
31. Hadlington JL, D. J. (2000) *Curr Opin Plant Biol.* **3**, 461-468.
32. Okamoto, T., Shimada, T., Hara-Nishimura, I., Nishimura, M., and Minamikawa, T. (2003) *Plant Physiol.* **132**, 1892-1900
33. Bardor, M., Faveeuw, C., Fitchette, A.-C., Gilbert, D., Galas, L., Trottein, F., Faye, L., and Lerouge, P. (2003) *Glycobiology* **13**, 427-434
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